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mcr-Positive *Escherichia coli* ST131-H22 from Poultry in Brazil

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Escherichia coli sequence type (ST) 131 is of concern because it can acquire antimicrobial resistance and cause extraintestinal infections. *E. coli* ST131-*H*22 sublineage appears capable of being transmitted to humans through poultry. We report on multidrug-resistant ST131-*H*22 poultry isolates in Brazil closely related to international human and poultry isolates.

The pandemic, extraintestinal, pathogenic *Escherichia coli* multilocus sequence type (MLST) 131 lineage has emerged extensively, gaining notoriety for its extensively multidrug-resistant ST131-*H*30 sublineage (*1*). Whereas ST131-*H*30 appears to be transmitted primarily from person to person, the *H*22 sublineage may be transmitted zoonotically through poultry and cause urinary tract infections and urosepsis (*2*,*3*). We report isolating ST131-*H*22 strains that are multidrug resistant (MDR), meaning that they are resistant to ≥3 classes of antimicrobials (*4*), carrying mobile colistin-resistance (*mcr*) determinants from poultry in Brazil, the largest poultry-exporting country in the world.

We collected 64 *E. coli* strains from poultry with colibacillosis cases from 2 different farms in the same geographic region of Brazil and screened them by PCR for the ST131 clonal group (*5*). PCR detected 6 ST131 isolates (2 from the first farm, 4 from the second), which we whole-genome sequenced (BioProject no. PRJNA398035). We determined phenotypic antimicrobial susceptibility with disk diffusion testing, except for isolates carrying the *mcr* gene, which we tested using broth microdilution (*6*).

We trimmed the reads and used QUAST (http://quast.sourceforge.net) to evaluate the quality of assemblies (contig lengths and expected genome sizes). We assembled DNA sequences with SPAdes (http://cab.spbu.ru/software/spades), then determined the serotype, phylogroup, MLST, *fimH* protein type, virulence gene profile, plasmid replicons, and markers of antimicrobial resistance for each isolate *in silico* using the ABRicate virulence factors database (https://github.com/tseemann/abricate) and ResFinder/PlasmidFinder tools from CGE (https://cge.cbs.dtu.dk/services). Genes were identified with a minimum of ≥95% of identity and coverage.

We identified all isolates as O25:H4-ST131-*H*22, all belonging to phylogroup B2. We generated a maximum-likelihood phylogeny tree on the basis of core-genome single-nucleotide polymorphisms, including the 6 isolates from Brazil and 140 ST131-*H*22 sequences from EnteroBase (http://enterobase.warwick.ac.uk) and a previous study (*2*), using the Northern Arizona SNP Pipeline (https://tgennorth.github.io/NASP/) aligned against *E. coli* JJ1886 ST131-*H*30 (GenBank

accession no. CP006784) (Appendix, https://wwwnc.cdc.gov/EID/article/26/8/19-1724-App1.pdf). The 6 isolates from poultry were nested within a clade of intermingled poultry and human clinical isolates within the overall international isolates (Figure, panel A). The isolates from Brazil were closely related to ST131-*H*22 avian pathogenic *E. coli* isolates from poultry in the United States and those from a human urinary tract infection in Australia (Figure, panel B). Identical virulence factors and plasmid replicons were observed among 4 β-lactamase positive isolates and between 2 isolates missing the β-lactamase genes but

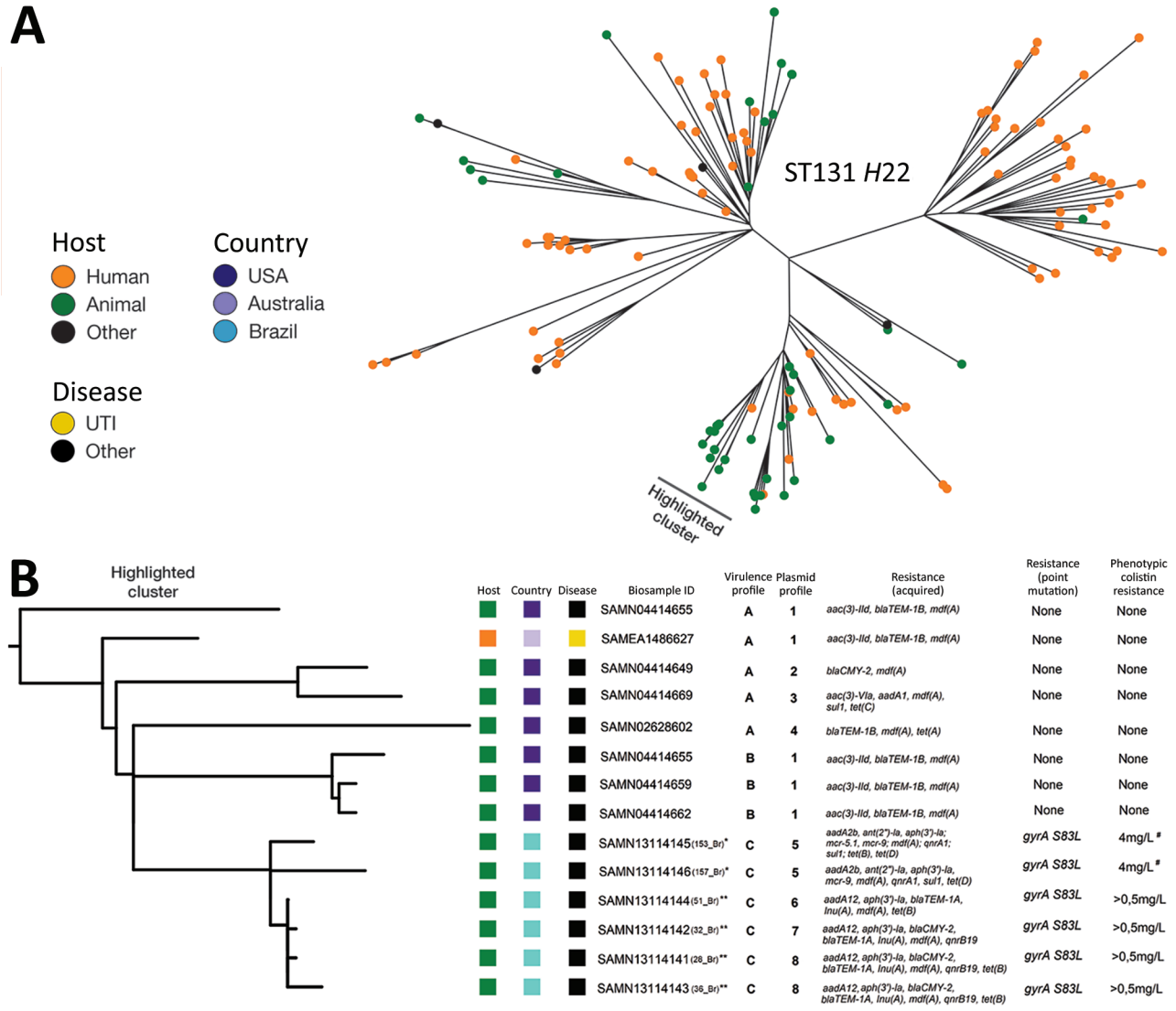

Figure. Phylogenetic analysis of *Escherichia coli* ST131-*H*22 isolates from poultry in Brazil and reference sequences. A) Unrooted phylogeny of 146 *E. coli* ST131-*H*22 isolates based on core genome single-nucleotide polymorphisms with the host origin outlined. The cluster containing closely related isolates to the 6 isolates from Brazil is highlighted. B) Rooted phylogeny of closely related isolates from retail meat with APEC and a human isolate with our 6 APEC isolates. The highlighted cluster includes a partial depiction of the tree including the data on host, country, and disease (urinary tract infection or other). Clusters containing the study's isolates have their individual identification in parenthesis. Asterisks indicate farm origins (*, farm 1; **, farm 2). Virulence factors profiles are identified as groups A: *cvi/cva, ent, fimA-H, ibeA, irp1/2, iroN, iucD,iss, kpsM, ompA, tsh;* B: *cvi/cva, ent, fimA-H, ibeA, irp1/2, iroN, iucD, iss, kpsM, ompA*; and C: *cvi/cva, ent, fimA-H, fyuA, ibeA, irp1/2, iroN, iucD, iss, kpsM, ompA, tsh*. Plasmid profiles are identified by group: 1: IncFIB, IncFIC(FII), IncI1; 2: IncFIB, IncFIC(FII), IncFII, IncI1; 3: IncFIB, IncFIC(FII), IncFII, IncHI2, IncHI2A, IncI1; 4: IncFIB, IncFIC(FII), IncI1, IncN; 5: IncFIB, IncFIC(FII), IncFII, IncFII(pCoo), IncHI2, IncHI2A; 6: IncFIB, IncFIC(FII), IncFII, IncHI2, IncHI2A; 7: IncFIB, IncFII, IncI1, IncX1; and 8: IncFIB, IncFIC(FII), IncFII, IncI1, IncX1. Phenotypic colistin-resistance is indicated by the symbol # for the 2 colistin-resistance *mcr* genes positive isolates, showing resistance according to 2018 Clinical Laboratory Standards Institute (https://clsi.org/) clinical breakpoints. APEC, avian pathogenic *E. coli*; ID, identification; ST, sequence type; UTI, urinary tract infection.

carrying *mcr* colistin–resistance determinants. All 6 isolates had MDR profiles, phenotypically confirmed (data not shown except for those from colistin microdilution method) (Figure, panel B).

The ST131-*H*22 lineage, while currently not as common as the *H*30 sublineage as a cause of community-acquired infections, does present a public health challenge because it colonizes poultry flocks, contaminating retail poultry products, and carries *mcr* colistin–resistance genes (*3*). The enormity and rapid growth of poultry production, in which many developing countries use antimicrobials extensively (*5*), and its zoonotic potential, make ST131-*H*22 worthy of specific attention (*2*).

Findings from our phylogenetic analyses of a global collection of ST131-*H*22 isolates from humans and poultry support findings from previous studies (*2*,*3*) and underscore the zoonotic potential of this virulent sublineage. Given that Brazil annually processes 13.8 million poultry products and exports 3.8 million kilograms (*4*), these findings warrant further examination to assess potential zoonotic spillover in Brazil and poultry-importing countries. Until such studies are conducted, the zoonotic potential of ST131-*H*22 in flocks in Brazil cannot be quantified.

The discovery of *mcr* mobile colistin resistance determinants in food animals has renewed attention to the potential risks of widespread antimicrobial use in livestock. In Latin America, *mcr-5* has been found in poultry in Paraguay (9). The description of the *mcr-9* homologue from humans in the United States and horses in Sweden has raised attention to another *mcr* gene with potential for global spread (*10*). Both *mcr* variants in this study, 153_Br and 157_Br, showed phenotypic resistance (*6*) and came from the same farm (Figure, panel B). Interestingly, 153_Br carried both *mcr-5.1* and *mcr-9* variants. These isolates may portend a more widespread problem within poultry flocks in Brazil.

Isolates from this study showed resistance to all of the World Health Organization's highest priority critically important antimicrobial classes (Figure, panel B) (*8*). Analysis of the absence of tetracycline resistance (*tet*[B]/[D]) in 1 of our isolates (Figure, panel B) indicates partial plasmid loss (data not shown).

Use of colistin as a growth promoter in livestock was banned in Brazil in November 2016, although it continued being therapeutically used in poultry up to 2018 (*7*). Therefore, *mcr*-encoding *H*22 strains could be selected out of the population over time. Further restrictions will have to be implemented to combat the growing resistance of *E. coli* in poultry in Brazil to critically important antimicrobial drugs (*4*).

Our findings suggest that poultry in Brazil may serve as a reservoir for MDR extraintestinal pathogenic *E. coli* carrying mobile colistin-resistance determinants. These findings highlight the need for better antimicrobial stewardship and surveillance systems to determine the prevalence of MDR *E. coli* ST131-*H*22 in these poultry flocks and clarify the risks posed to domestic and international poultry consumers.

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Heartland Virus in Lone Star Ticks, Alabama, USA

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We detected Heartland virus (HRTV) in lone star nymphs collected in 2018 in northern Alabama, USA. Real-time reverse transcription PCR selective for the small segment of the HRTV genome and confirmatory sequencing of positive samples showed high identity with HRTV strains sequenced from Tennessee and Missouri.

Heartland virus (HRTV) is an emerging pathogenic phlebovirus first identified in the United States in 2009 and now reported in 15 states (*1*,*2*). Nymphal lone star ticks (*Amblyomma americanum*) are considered the primary vectors of HRTV, and a variety of domestic and endemic mammalian species are potential amplification hosts of this virus (*2*,*3*). Although *A. americanum* ticks are well-established throughout the eastern, southeastern, and midwestern United States, their range is expanding northward and westward, most likely because of increased host availability and abundance, changes in environmental and climatic conditions, and adaptive genetic variation (Figure, panel A) (*4*). We tested for HRTV in *A. americanum* ticks collected in Alabama, USA, a state within the range of this vector where HRTV has not been documented previously from ticks.

From June 1, 2018, through August 31, 2018, we collected ticks as previously described (*5*) in the William B. Bankhead National Forest, Alabama (34.2270°N, 87.3461°W; Figure, panel B). In preparation for pathogen screening, we separated ticks into pools. Nymph tick pools ranged from 1 to 5 tick(s) of the same species per pool. We screened adult ticks individually (i.e., 1 adult tick per pool) (Appendix Table, https://wwwnc.cdc.gov/EID/article/26/8/19-0494-App1.pdf). We did not include larvae in pathogen screening. We used molecular methods to extract viral RNA and detect the small (S) segment of the HRTV genome using the HRTV-4 primer and probe set (*6*) in tick pools (Appendix Table). We sequenced HRTV-4–positive samples using the Ion Torrent Personal Genomic Machine system (Life Technologies, https://www.thermofisher.com) at the Centers for Disease Control and Prevention (CDC; Fort Collins, CO, USA) as described previously (*7*). We obtained sequences of the HTRV S segment of other HRTV samples and strains from the GenBank database, and aligned sequences using the MUSCLE alignment tool (https://www.ebi.ac.uk/Tools/msa/muscle) in MEGA software (*8*). We also included a closely related severe fever with thrombocytopenia syndrome virus isolate from the GenBank database as an outgroup for this analysis. We used a maximum-likelihood tree approach with 1,000 bootstrap replications to generate the genetic relationships between the Alabama samples and the other HRTV samples available through the GenBank database.

We collected 964 ticks, of which 921 were *A. americanum* (872 nymphs, 22 adult males, and 27 adult females) and 43 were *Dermacentor variabilis* (20 adult males and 23 adult females). We tested

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mcr-Positive *Escherichia coli* ST131-*H22* from Poultry Connected to International Isolates, Brazil

Appendix

Appendix Table. ST131-*H*22 genomic sequences (N = 140) obtained from GenBank and EnteroBase repositories and the 6 Brazilian poultry isolates from this study, with added metadata on host, continent, country of isolation, and disease, when available

Accession numbers (Biosample IDs)	Host	Continent	Country	Disease
SAMN04414658	Poultry	North America	United States	Other/food
SAMN04414651	Poultry	North America	United States	Other/food
SAMN04414654	Poultry	North America	United States	Other/food
SAMN04414650	Poultry	North America	United States	Other/food
SAMN04414646	Poultry	North America	United States	Other/food
SAMN04414652	Poultry	North America	United States	Other/food
SAMN04414653	Poultry	North America	United States	Other/food
SAMN04414671	Poultry	North America	United States	Other/food
SAMN04414657	Poultry	North America	United States	Other/food
SAMN04414698	Human	North America	United States	Urinary tract infection
SAMN04414684	Human	North America	United States	Urinary tract infection
SAMN04414818	Human	North America	United States	Urinary tract infection
SAMN04414670	Poultry	North America	United States	Other/food
SAMN04414668	Poultry	North America	United States	Other/food
SAMN04414799	Human	North America	United States	Urinary tract infection
SAMN04414648	Poultry	North America	United States	Other/food
SAMN04414645	Poultry	North America	United States	Other/food
SAMN04414660	Poultry	North America	United States	Other/food
SAMN04414655	Poultry	North America	United States	Other/food
SAMN04414659	Poultry	North America	United States	Other/food
SAMN04414662	Poultry	North America	United States	Other/food
SAMN04414649	Poultry	North America	United States	Other/food
SAMN04414669	Poultry	North America	United States	Other/food
SAMN04414647	Poultry	North America	United States	Other/food
SAMN04414661	Poultry	North America	United States	Other/food
SAMN04414667	Poultry	North America	United States	Other/food
SAMN04414678	Human	North America	United States	Urinary tract infection
SAMN04414779	Human	North America	United States	Urinary tract infection
SAMN04414780	Human	North America	United States	Urinary tract infection
SAMN04414676	Human	North America	United States	Urinary tract infection
SAMN04414790	Human	North America	United States	Urinary tract infection
SAMN04414797	Human	North America	United States	Urinary tract infection
SAMN04414700	Human	North America	United States	Urinary tract infection
SAMN04414767	Human	North America	United States	Urinary tract infection
SAMN04414798	Human	North America	United States	Urinary tract infection
SAMN04414738	Human	North America	United States	Urinary tract infection
SAMN04414761	Human	North America	United States	Urinary tract infection
SAMN04414793	Human	North America	United States	Urinary tract infection
SAMN04414844	Human	North America	United States	Urinary tract infection
SAMN04414827	Human	North America	United States	Urinary tract infection
SAMN04414822	Human	North America	United States	Urinary tract infection
SAMN04414810	Human	North America	United States	Urinary tract infection
SAMN04414816	Human	North America	United States	Urinary tract infection
SAMN04414831	Human	North America	United States	Urinary tract infection
SAMN04414809	Human	North America	United States	Urinary tract infection
SAMN04414848	Human	North America	United States	Urinary tract infection
SAMN07679510	Poultry	North America	United States	Other/food
SAMN04414665	Poultry	North America	United States	Other/food

Accession numbers (Biosample IDs)	Host	Continent	Country	Disease
SAMN04414663	Poultry	North America	United States	Other/food
SAMN02228508	Poultry	North America	United States	Colibacillosis
SAMN04992373	Poultry	North America	United States	Colibacillosis
SAMN02628602	Poultry	North America	United States	Other/food
SAMN02628600	Poultry	North America	United States	Other/food
SAMN02628555	Poultry	North America	United States	Other/food
SAMN02628549	Poultry	North America	United States	Other/food
SAMN02628547	Poultry	North America	United States	Other/food
SAMN02442811	Poultry	North America	United States	Other/food
SAMN02228511	Poultry	North America	United States	Colibacillosis
SAMN01885791	Human	Europe	Denmark	Urosepsis
SAMN01885786	Human	Europe	Denmark	Urosepsis
SAMN01885680	Human	Europe	Denmark	Urosepsis
SAMEA1486636	Human	North America	Canada	Septicemia
SAMEA1486663	Human	Europe	Spain	Urinary tract infection
SAMEA1486626	Human	Europe	Spain	Urosepsis
SAMEA1486672	Human	Europe	Spain	Urinary tract infection
SAMEA1486674	Human	Europe	Spain	Urinary tract infection
SAMEA1486627	Human	Oceania	Australia	Urinary tract infection
SAMN04159648	Human	Europe	Germany	Respiratory infection
SAMEA3712539	Human	Europe	Denmark	Other/not available
SAMN02603887*	Human	North America	United States	Reference strain for alignment
SAMN05170667	Human	Europe	United Kingdom	Other/not available
SAMN04273082	Human	Europe	United Kingdom	Septicemia
SAMN04273074	Human	Europe	Germany	Urinary tract infection
SAMN04273073	Human	Europe	Germany	Urinary tract infection
SAMN04357670	Human	Europe	United Kingdom	Septicemia
SAMEA2500548	Human	Europe	Spain	Abscess
SAMN05170071	Human	North America	United States	Urinary tract infection
SAMN05567350	Human	Oceania	Australia	Other/Feces
SAMEA3268827	Swine	Europe	Denmark	Other/Feces
SAMN02228459	Poultry	North America	United States	Other/not available
SAMN02228457	Poultry	North America	United States	Other/not available
SAMN02228456	Poultry	North America	United States	Other/not available
SAMN02442854	Poultry	North America	United States	Other/food
SAMN02801921	Human	North America	United States	Urinary tract infection
SAMN01885818	Human	Europe	Denmark	Urosepsis
SAMN01885841	Human	Europe	Denmark	Urosepsis
SAMN01885739	Human	Europe	Denmark	Urosepsis
SAMEA1486661	Human	Oceania	Australia	Urinary tract infection
SAMEA1486587	Human	Oceania	Australia	Urinary tract infection
SAMEA1486667	Human	Oceania	Australia	Urinary tract infection
SAMEA1486588	Human	Europe	United Kingdom	Urinary tract infection
SAMEA1486633	Human	Europe	United Kingdom	Urinary tract infection
SAMEA1486650	Human	Europe	United Kingdom	Urinary tract infection
SAMEA1486631	Human	Europe	United Kingdom	Urinary tract infection
SAMEA1486670	Human	Europe	Spain	Abdominal abscess
SAMEA1486601	Human	Europe	United Kingdom	Urinary tract infection
SAMEA1486662	Human	Europe	United Kingdom	Urinary tract infection
SAMN02228548	Human	Oceania	Australia	Other/not available
SAMN02228503	Human	North America	United States	Other/not available
SAMN02228498	Human	North America	United States	Other/not available
SAMN02228469	Human	North America	United States	Other/not available
SAMN02228455	Human	North America	United States	Other/not available
SAMN02228454	Human	Asia	India	Other/not available
SAMN02228453	Human	North America	United States	Other/not available
SAMN04159621	Human	Asia	Cambodia	Urinary tract infection
SAMN04159620	Human	Asia	Cambodia	Urinary tract infection
SAMN04159601	Human	Asia	Cambodia	Urinary tract infection
SAMN04159599	Human	Asia	Cambodia	Septicemia
SAMN04159570	Human	Europe	United Kingdom	Septicemia
SAMN04159569	Human	Europe	United Kingdom	Septicemia
SAMN02228503	Human	North America	Canada	Other/not available
SAMN02228496	Human	North America	United States	Other/not available
SAMN02228471	Human	North America	United States	Other/Environment
SAMN05163698	Human	Europe	United Kingdom	Other/not available
SAMN04273126	Human	Europe	United Kingdom	Urinary tract infection
SAMN04273123	Human	Europe	United Kingdom	Urinary tract infection

Accession numbers (Biosample IDs)	Host	Continent	Country	Disease
SAMN04273121	Human	Europe	Ireland	Urinary tract infection
SAMN04273096	Human	Europe	United Kingdom	Septicemia
SAMN04273095	Human	Europe	United Kingdom	Septicemia
SAMN04273093	Human	Europe	United Kingdom	Septicemia
SAMN04273090	Human	Europe	United Kingdom	Septicemia
SAMN04273088	Human	Europe	United Kingdom	Septicemia
SAMN04273067	Human	Europe	Germany	Urinary tract infection
SAMN04273066	Human	Europe	Germany	Urinary tract infection
SAMN04273061	Human	Europe	Germany	Urinary tract infection
SAMN03922994	Human	North America	United States	Septicemia
SAMN04357658	Human	Europe	United Kingdom	Septicemia
SAMEA1094703	Human	Europe	United Kingdom	Urinary tract infection
SAMN05170071	Human	North America	United States	Urinary tract infection
SAMD00044939	Human	Asia	Japan	Other/not available
SAMD00044931	Human	Asia	Japan	Other/not available
SAMEA3180298	Human	Europe	Germany	Other/not available
SAMN05567349	Human	Oceania	Australia	Feces
SAMN02228458	Human	North America	United States	Other/not available
SAMN02228472	Primate	North America	United States	Other/not available
SAMN04992249	Swine	North America	United States	Other/not available
SAMN02228471	Water	North America	United States	Other/Environment
SAMN05440287	Water	North America	United States	Other/Environment
SAMEA104402259	Human	South America	Brazil	Urinary tract infection
SAMEA104402296	Human	South America	Brazil	Urinary tract infection
SAMN13114141	Poultry	South America	Brazil	Colibacillosis
SAMN13114142	Poultry	South America	Brazil	Colibacillosis
SAMN13114143	Poultry	South America	Brazil	Colibacillosis
SAMN13114144	Poultry	South America	Brazil	Colibacillosis
SAMN13114145	Poultry	South America	Brazil	Colibacillosis
SAMN13114146	Poultry	South America	Brazil	Colibacillosis

*Reference strain *Escherichia coli* ST131 isolate JJ1886.